Antiobesity effects of KR-66195, a synthetic DPP-IV inhibitor in diet-induced obese mice and obese-diabetic *ob/ob* mice

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Directed by Professor Hyun Chul Lee

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This certifies that the Master's Thesis of
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<ABSTRACT>

Antiobesity effects of KR-66195, a synthetic DPP-IV inhibitor in diet-induced obese mice and obese-diabetic *ob/ob* mice

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A variety of dipeptidyl peptidase (DPP)-IV inhibitors showed improved glycemic control in patients with type 2 diabetes by increasing plasma level of active glucagon like peptide (GLP)–1. However, these DPP-IV inhibitors failed to show weight reduction. In this study, we found KR-66195, a new DPP-IV inhibitor could show preventing weight gain effects as well as improved glycemic control in both diet-induced obese C57BL/6 mice and obese-diabetic *ob/ob* mice. In C57BL/6 mice, intraperitoneal administration of KR-66195 for 8 weeks at 10 mg per kg of body weight once a day resulted in increase in the amount of plasma GLP-1, improvement of glucose tolerance and reduction in body weight gain, epididymal fat accumulation and food intake. In *ob/ob* mice, the same administration of KR-66195 for 3 weeks

resulted in the comparable effects as well as increased pancreatic insulin content. These results suggest that KR-66195 could be further developed as a therapeutic medication to treat obesity as well as diabetes.

Key words : obesity, diabetes, KR-66195, glucagon like peptide (GLP)-1, dipeptidyl peptidase (DPP)-IV, weight loss, diet-induced obesity, *ob/ob*, mice

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I. INTRODUCTION

Obesity is a major public health problem worldwide and is associated with significant morbidity and mortality. Ninety percent of patients with type 2 diabetes are overweight or obese.[1] It has been reported that obese patients who lose 5~10% of their initial body weight will have an improvement in insulin resistance and cardiovascular risk factors.[2]

However, weight management is more difficult in obese patients with type 2 diabetes and even more so in patients treated with sulphonylureas, thiazolidinediones or insulin therapy.[3-6] Weight loss agents, which are sibutramine, orlistat and remonabant, recently licensed for the treatment of obesity lead to statistically and clinically significant improvements in glycaemic parmeters and glycaemic control in patients with and without

diabetes.[7] But orlistat only has been reported to reduce the incidence of diabetes in subjects with impaired glucose tolerance. [7] Moreover, their side effects make it difficult to use in obese patients with type 2 diabetes.

In addition, it has been reported that the incretin effect might be partly impaired due to a reduction in glucagon-like peptide (GLP)-1 secretion in patients with type 2 diabetes.[8-9] GLP-1 secreted by gut endocrine cells stimulate glucose-dependent insulin secretion after nutrient ingestion and exert direct trophic effects on beta-cell proliferation, differentiation, growth and survival in vitro and in animal models of diabetes.[10-12] Furthermore, GLP-1 inhibits glucose-dependent glucagon secretion and has been reported to delay gastric emptying, enhance satiety and reduce food intake.[13] So it was thought that GLP-1 would be desirable drug for obese patients with diabetes.

However, the therapeutic potentials of GLP-1 are limited by their rapid degradation and inactivation in vivo by dipeptidyl peptidase (DPP)-IV.[14] Thus, degradation-resistant GLP-1 receptor agonists and DPP-IV inhibitors have come into view as new classes of pharmacological agents to enhance incretin action and thereby improve glycemic control in patients with type 2 diabetes.[15-17] Studies have demonstrated the ability of these agents to improve glycemic control in animal models of diabetes and in patients with type 2 diabetes.[18-20] Accumulating evidences suggest that exogenously administered GLP-1 receptor agonists show trophic effects on pancreatic islets in animal models of diabetes, increasing islet neogenesis and differentiation as well as

increasing beta-cell mass.[21-24] On the contrary, considerably scant data exist on in vivo effects of chronic DPP-IV inhibition on pancreatic islets, even less on antiobesity effects.[25-26] Recent studies have shown that chronic treatment with sitagliptin, an analogue of the DPP-IV inhibitor increased islet insulin content and improved responsiveness of islets to glucose-stimulated insulin secretion in high-fat-diet fed streptozotocin mice.[27] But these studies did not show weight reduction or prevention relating to antiobesity effect.

The aim of the present studies was to investigate long term effects of KR-66195, a recently developed DPP-IV inhibitor, in diet-induced obese C57BL/6 for 8 weeks and short term effects in obese-diabetic *ob/ob* mice for 3 weeks. KR-66195 would inhibit GLP-1 degradation by DPP-IV inhibition and might cause weight loss or inhibit weight gain because of its prolonged activation time.

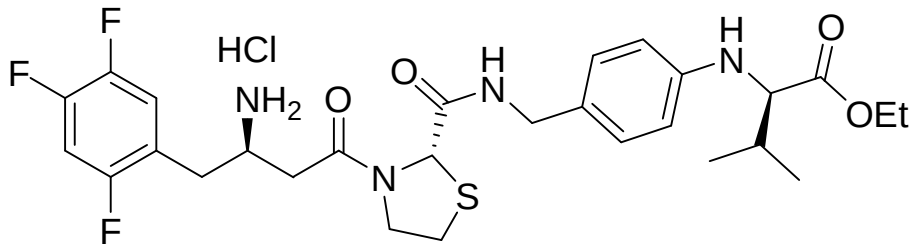


Figure 1. Chemical structure of KR-66195

II. MATERIALS AND METHODS

1. Chemicals

KR-66195 (novel 2-carbonyl-3-acyl-1,3-thiazolindies derivertives) was synthesized and provided by drug discovery division in Korea Research Institute of Chemical Technology (KRICT) (Taejon, Korea). (Fig. 1) Doses of the KR-66195 chosen based on literature review and pilot studies in KRICT. KR-66195 was diluted in Phosphate buffered saline (PBS) with 2 mg/ml concentration, then filtered with Millex-GV (Millipore, MA, USA). KR-66195 injected with 10 mg/kg (chemical/body weight) concentration using BD ultra fine II insulin syringe with 31 guage needles (Becton, Dickinson and Company, NJ, USA). All reagents were purchased from Sigma-Aldrich (MO, USA).

2. long term study in C57BL/6 mice

Male C57BL/6 mice were purchased from at SLC (Tokyo, Japan) at five weeks of age. All mice were housed under specific pathogen–free conditions in individual cages and maintained on a 12 h light / dark cycle with continuous access to food and water. They were acclimatized to the Yonsei University Health System Clinical Trial Center for 2 weeks, during which time they were fed a normal chow diet (CHD). CHD was Formulab diet 5008 (Purina LabDiet, IN, USA), which contains 6.5% fat by weight and provides 16.7% total calories from fat. At 8 weeks of age, mice are divided into two

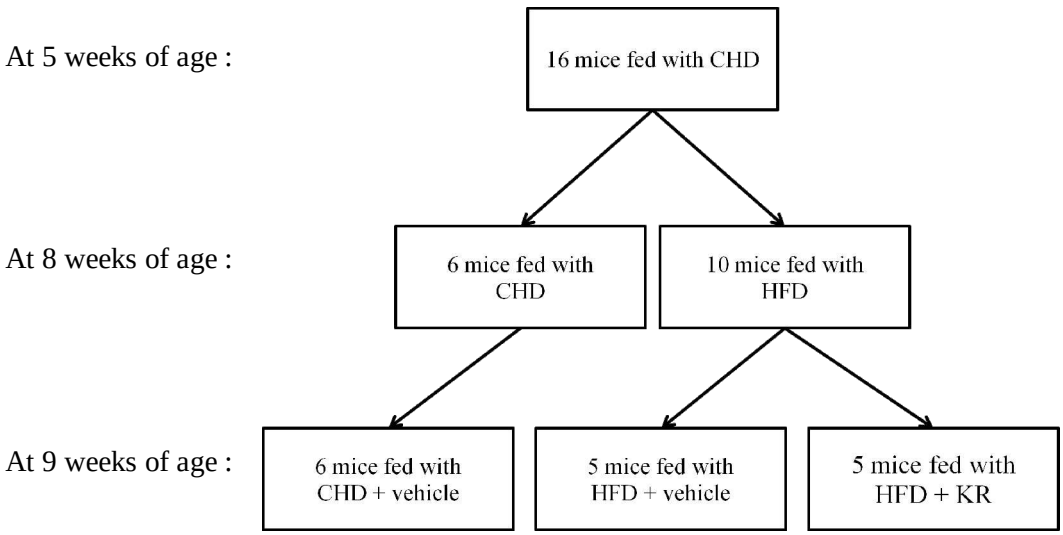
groups: CHD group (N=6) and high fat diet (HFD) group (N=10). HFD was D12451 (Research Diets, NJ, USA), which contains 24% fat by weight and provides 45% total calories from fat. 1 week after commencement of HFD, the group was also divided into 2 further groups: HFD receiving vehicle (PBS) injection group (HFD+vehicle) (n=5) and HFD receiving KR-66195 injection group (HFD+KR) (n=5). All groups, CHD+vehicle, HFD+vehicle and HFD+KR, were treated with once-daily intraperitoneal (IP) injections of 10mg/kg KR-66195 with vehicle or vehicle alone. Mice were housed in a single cage. After the mice were injected, body weight and food intake were monitored 2-3 times per week. After 8 weeks of treatment, glucose tolerance was assessed by intraperitoneal glucose tolerance test (IPGTT). The study was completed after 8 weeks of drug treatment and mice were sacrificed 1 day after final drug treatment.

3. Short term study in *ob/ob* mice

Female *ob/ob* mice were purchased from Charles River Laboratories (PQ, Canada) at seven weeks of age. All mice were housed under specific pathogen–free conditions in individual cages and maintained on a 12 h light / dark cycle with continuous access to food and water. They were acclimatized to the Yonsei University Health System Clinical Trial Center for 1 week, during which time they were fed with CHD. At 8 weeks of age, they were divided into 2 groups; KR (N=6) and Vehicle (N=6). All groups, KR and

Vehicle, were treated with once-daily IP injections of 10 mg/kg KR-66195 with vehicle or vehicle alone. After the mice were injected, body weight and food intake were monitored 2-3 times per week. Glucose tolerance was assessed by IPGTT weekly. The study was completed after 3 weeks of drug treatment and the mice were sacrificed 2 hour after the final drug treatment.

(A)



(B)

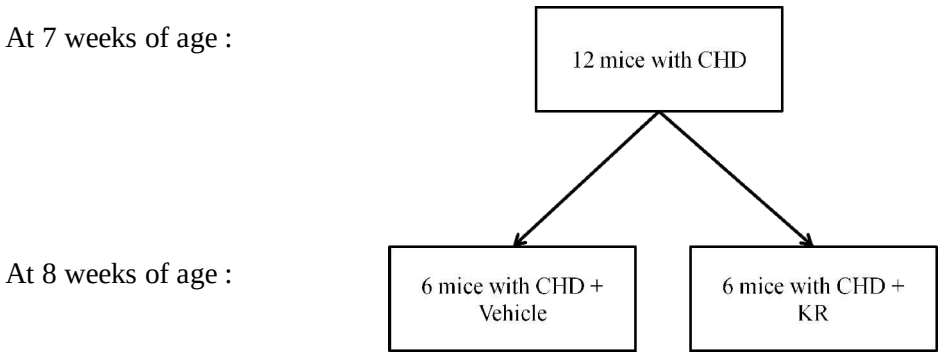


Figure 2. Study design of (A) C57BL/6 and (B) *ob/ob* mice

3. Glucose tolerance test

Mice were fasted overnight and treated with IP glucose injection (2 g/kg body wt). Tail blood was collected at 0, 15, 30, 60, 120 min. Blood glucose concentration was measured using a Accu-Chek Active (Roche, Basel, Switzerland). In *ob/ob* mice study, blood glucose concentration was assayed after dilution with PBS because of high glucose concentration (higher than 600 mg/dl).

4. Plasma analysis

Cardiac blood was collected in EDTA tubes (Sarstedt, Nümbrecht, Germany) for GLP-1 analysis immediately after sacrifice. After centrifugation for 10 minutes at 1500g, plasma was stored at -20C. Concentrations of GLP-1 (7-36) amide were measured with an active GLP-1 ELISA Kit (Linco Research, MO, USA).

5. Quantitation of islet area

Pancreatic sections (4 μm) were incubated overnight at 4°C with guinea pig anti-insulin antibody (Dako Diagnostics, Ontario, Canada). The samples were then incubated for 1 h with biotinylated anti-guinea pig antibody (Vector Laboratories, Ontario, Canada), and subsequently treated for 1 h with avidin/biotin complex (Vectastain Elite ABC Kit; Vector Laboratories, CA, USA). Sections were then stained with 3,3′-diaminobenzidine

tetrahydrochloride (DAB) for 10 min. After DAB staining, the sections were washed with tap water and counterstained with hematoxylin. Three sections of whole pancreas from each animal were scanned by Dotslide virtual microscope (Olympus, Tokyo, Japan) following insulin immuno-histochemistry. The relative islet area was determined by quantification of the cross-sectional area occupied by beta-cells and the total pancreatic cross-sectional area with Metamorph (Universal Imaging, PA, USA) image analysis software.

6. Statistics

Statistical analyses were performed with the Student's t test for independent samples (nonparametric), and data are expressed as means ± standard error mean (SE) or standard deviation (SD) unless. A P value of <0.05 was considered as statistically significant.

III. RESULTS

Studies in C57BL/6 mice

1. Long term KR-66195 injection prevented C57BL/6 mice from diet-induced obesity

Before initiation of the treatment, it was confirmed that there were no significant differences between groups of mice allocated to the different treatment groups in food intake or weight. To determine the effects of KR-66195 on body weight, animals were divided into three groups at 9 weeks of age (CHD+vehicle: n=6, HFD+vehicle: n=5, HFD+KR: n=5). HFD+KR group exhibited significantly less body weight gain over time than HF+vehicle group, and the trend was sustained over the entire treatment period (Fig. 3A). As expected, the HFD+vehicle group gained substantially more body weight than CHD+vehicle group. But HFD+KR group gain less weight than HFD+vehicle group. After 8 weeks of drug treatment, HFD+KR group gained 5.38±1.89g, HFD+vehicle group gained 12.08±1.36g and CHD+vehicle group gained 3.67±2.89g <Table 1>. To examine the mechanisms underlying the reduced weight gain in the face of an energy-rich diet, we measured average food intake. KR-66195 treated mice (2.41 ± 0.09 g) consumed significantly less food daily than vehicle treated mice (2.79 ± 0.11 g) over the 8-week treatment period ($P < 0.05$) (Fig. 3B). And the reduced body weight gain in high fat–fed mice treated with KR resulted in significant

reductions in epididymal fat mass ratio (g organ weight / g body weight) (Fig. 3C). The epididymal fat mass ratio was 0.025 ± 0.0015 in CHD+Vehicle group, 0.063 ± 0.0033 in HFD+vehicle group and 0.029 ± 0.0021 in HFD+KR group.

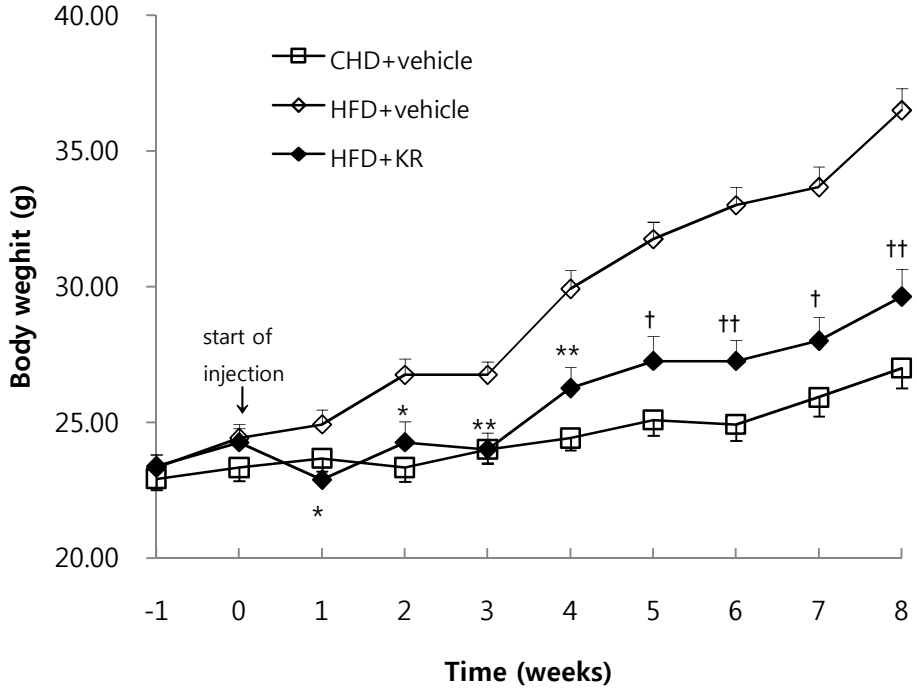


Figure 3A. KR-66195 reduced weight gain in diet-induced obese C57/BL6 mice. Weekly body weight in C57BL/6 mice treated with 10mg/kg KR-66195 with vehicle or vehicle alone once daily by IP injection for 8 weeks. Values are expressed as means ± SE; n=5-6 mice per group. *, $P < 0.05$; **, $P < 0.01$; †, $P < 0.005$; and ††, $P < 0.001$ vs. HFD+vehicle.

Table 1. Bodyweight at the baseline and final in C57BL/6 mice.

	Baseline weight	Final weight	Difference
CHD+vehicle	23.33±1.21 g	27.00±1.84 g	3.67±2.89 g
HFD+vehicle	24.42±1.24 g	36.50±1.97 g *	12.08±1.36 g
HFD+KR	24.25±1.04 g	29.63±2.06 g ††	5.38±1.89 g

Difference means difference between baseline weight and final weight. Values are expressed as means ± SD. ††P < 0.001 vs. HFD+vehicle; *, P < 0.05 vs. CHD+vehicle

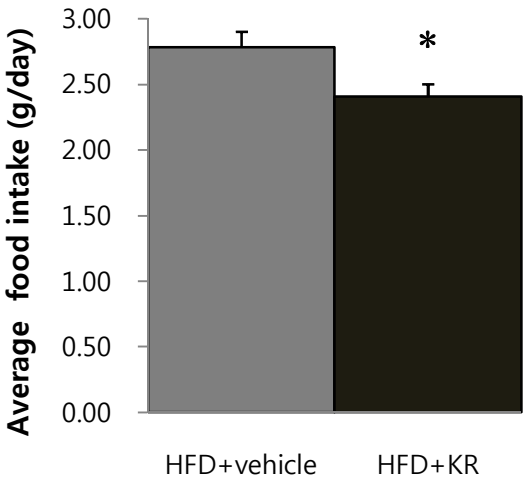
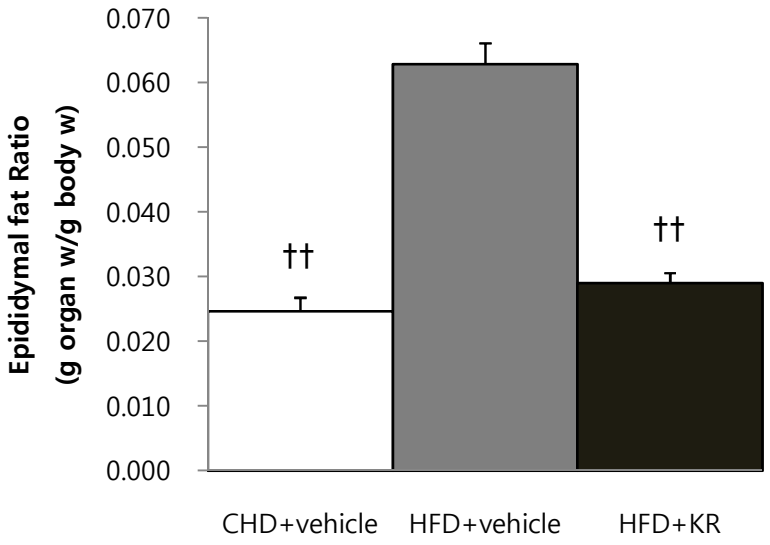


Figure 3B. Average food intake for 8 weeks. 10mg/kg KR-66195 treatment reduced average high-fat food consumption in C57BL/6 mice for 8 weeks. Values are expressed as means ± SE. *, P < 0.05 vs. HFD+vehicle.



Fugure 3C. Epididymal fat ratio of mice. 10mg/kg KR-66195 treatment for 8 weeks prevented epididymal fat accumulation in HFD+KR group. Values are expressed as means ± SE. ††, $P < 0.001$ vs HFD+vehicle.

2. KR-66195 improved glucose tolerance in C57BL/6 mice

After 8 weeks of treatment in long-term study, the mice were not injected any drug for 40 hours before GTT, fasted for a further 15 h, and were administered glucose via IP injection, When glucose was administered, KR-66195 administered group showed significantly reduced the area under the glucose curve (AUC) compared with HFD+vehicle treated mice (Fig. 4A and 4B). In HFD+KR group, plateau blood glucose level was 440.25 ± 53.47 mg/dl at 30 min, but 500.83 ± 52.29 mg/dl at 60 min in HFD+vehicle group, and 324.33 ± 52.18 mg/dl at 15 min in CHD+vehicle group (Fig. 4A).

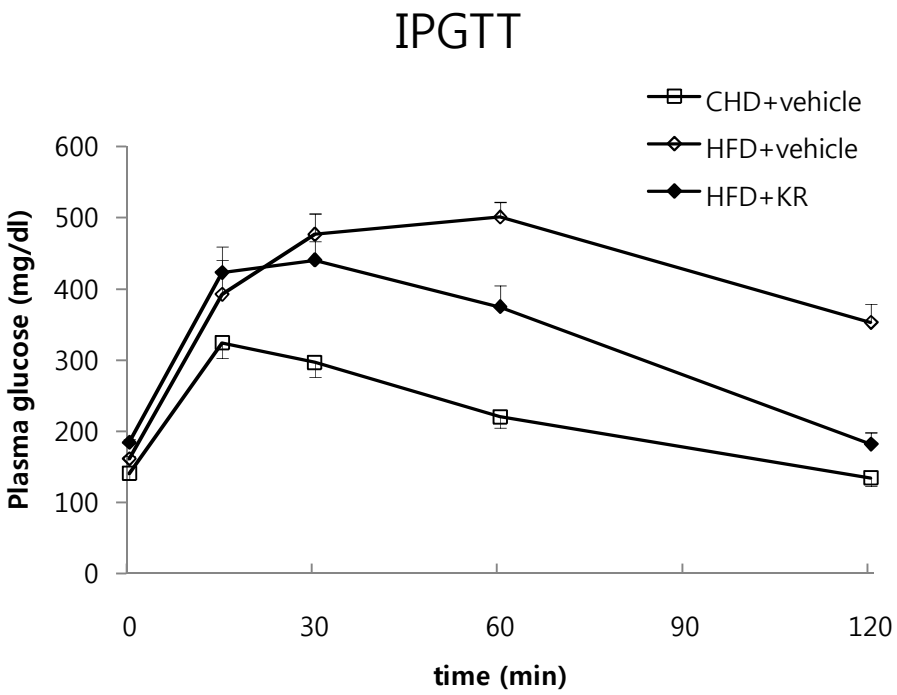


Figure 4A. C57BL/6 mice administrated KR-66195 for 8 weeks showed improved glucose tolerance during IPGTT. The mice administered KR-66195 for 8 weeks showed lowered plateau blood glucose level and improved blood glucose tolerance during IPGTT (2g glucose/kg body wt). Values are expressed as means ± SE; n=5-6 mice/group.

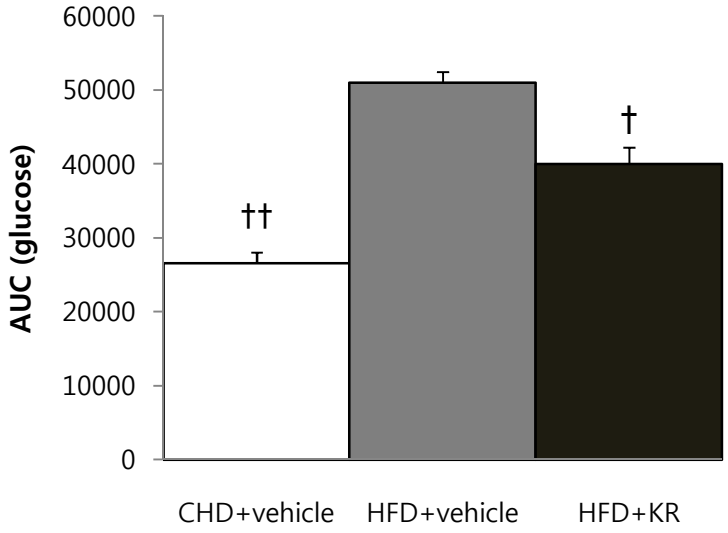


Figure 4B. AUC of IPGTT. AUC for the IPGTTs was calculated from 0 to 120 min. Values are expressed as means ± SE. †, $P < 0.005$; ††, $P < 0.001$ vs. HFD+vehicle.

3. KR-66195 increased plasma GLP-1 levels in C57BL/6 mice

After 8 weeks of treatment, blood was collected during sacrifice. Long-term treatment with KR-66195 increased basal GLP-1 plasma concentration to 14.70 ± 3.28 pM in HFD+KR group, on the other hands, 6.00 ± 0.75 pM in HFD+vehicle group and 7.86 ± 0.19 pM in CHD+vehicle group (Fig. 5).

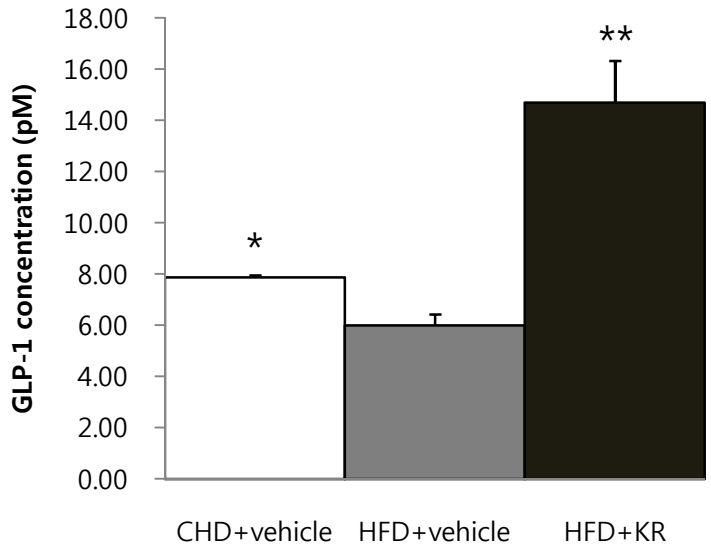


Figure 5. KR-66195 increased plasma GLP-1 concentration in C57BL/6 mice. KR-66195 treatment for 8 weeks increased plasma active GLP-1 levels. Values are expressed as means ± SE; n=5-6 mice per group. *, $P < 0.05$; **, $P < 0.01$ vs. HFD+vehicle.

Studies in *ob/ob* mice

4. Short-term injection of KR-66195 effectively prevented weight-gain in *ob/ob* mice

At the beginning of the experiment, initial body weight was higher in KR group (32.10 ± 3.16) than Vehicle group (30.88 ± 3.40g), but the difference was statistically insignificant (Fig. 6A). To determine the effects of KR-66195 on body weight, animals were divided into two groups at 8 weeks of age (Vehicle: n=6, KR: n=6). KR group exhibited significantly less body weight gain over time than Vehicle group, and the trend was sustained over the entire treatment period (Fig. 6A). In KR group, cumulative body weight gain was significantly smaller than that of Vehicle group <Table 2>. Moreover, the body weight of several mice in KR group was slightly reduced during the experiment. It was consistent with the study in C57BL/6 mice. After 3 weeks of drug treatment, KR group gained 1.06±3.76g, Vehicle group gained 10.78±2.51 g <Table 2>. And KR group (2.65±0.92g) consumed significantly less food daily than Vehicle group (5.23±0.48g) ($P<0.001$) (Fig. 6B).

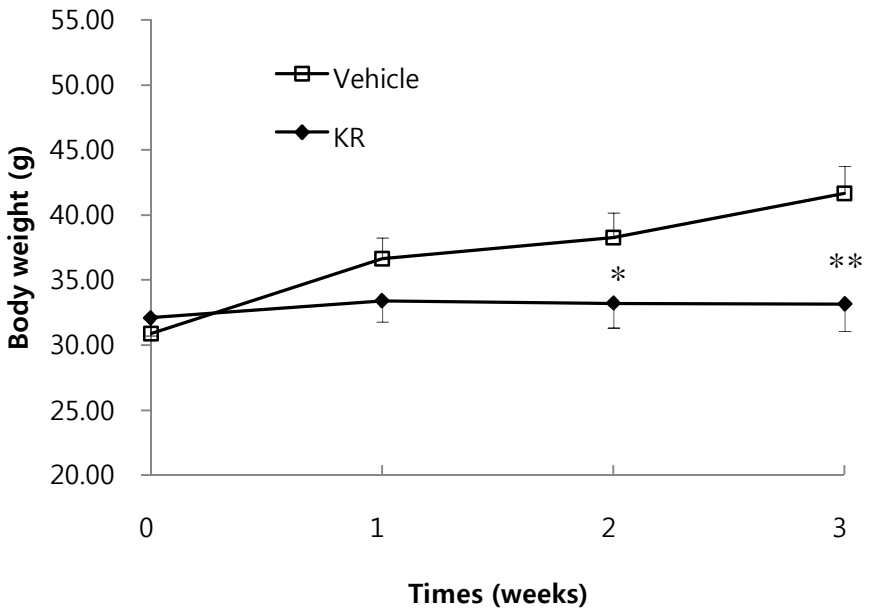


Figure 6A. KR-66195 prevented weight gain in *ob/ob* mice. Weekly body weight in *ob/ob* mice treated with 10mg/kg KR-66195 with vehicle or vehicle alone once daily by IP injection for 3 weeks. Values are expressed as means ± SE; n=6 mice per group. *,P < 0.05; **,P < 0.01 vs. Vehicle.

Table 2. Bodyweight at the baseline and final in *ob/ob* mice.

	baseline weight	final weight	Difference
Vehicle	30.88 ± 3.40 g	41.67 ± 5.14 g	10.78 ± 2.51 g
KR	32.10 ± 3.16 g	33.16 ± 2.97 g **	1.06 ± 3.76 g

Difference means difference between baseline weight and final weight. Values are expressed as means ± SD. **, P < 0.01 vs. Vehicle.

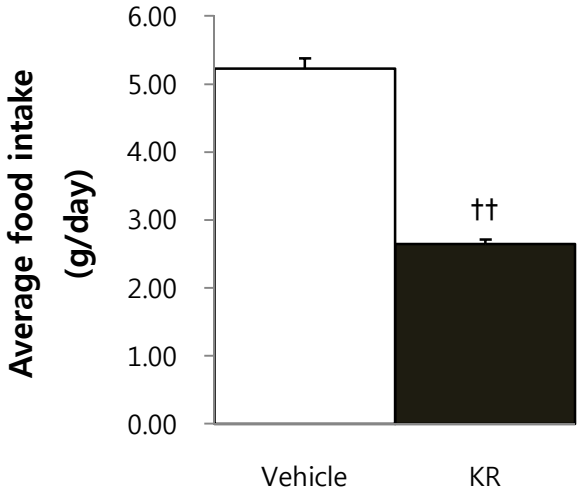
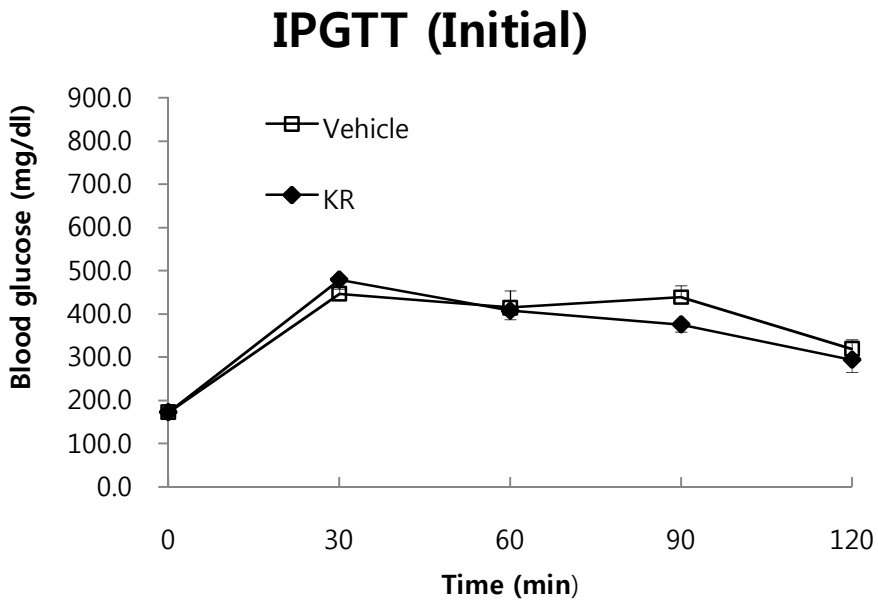


Figure 6B. Average food intake for 3 weeks. 10mg/kg KR-66195 treatment reduced average food consumption in *ob/ob* mice for 3 weeks. Values are expressed as means ± SE; n=6 mice/group. ††, $P < 0.001$ vs. Vehicle.

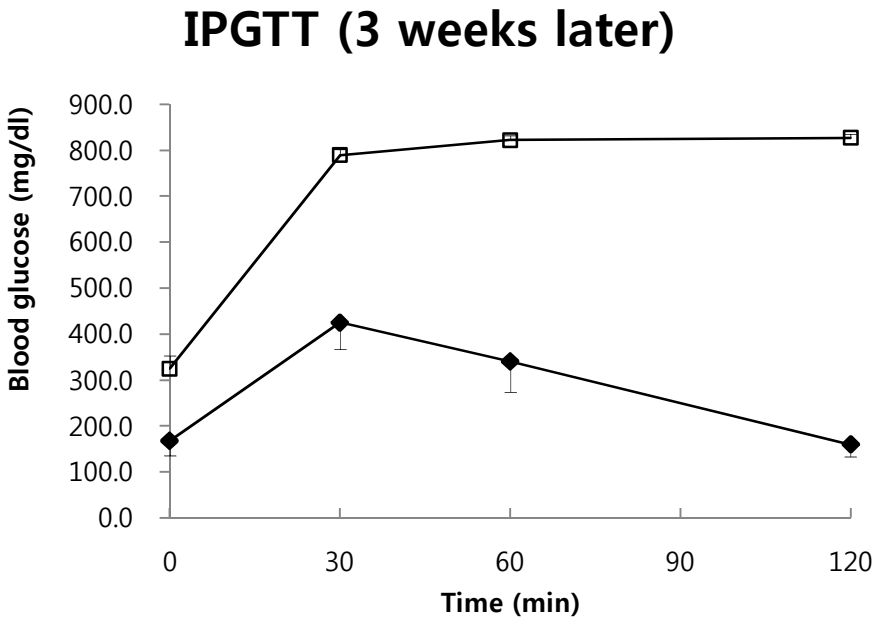
5. KR-66195 improved glucose tolerance in *ob/ob* mice

At the beginning of the study, two groups showed similar glucose excursion profile when glucose was administered via an IP injection (Fig. 7A and 7B). But, after 3 weeks of treatment, the treatment with KR-66195 prevented hyperglycemia in ob/ob mice. After 3 weeks of KR-66195 treatment, KR group showed similar glucose excursion profile with that of the initial, moreover, glucose clearance ability of KR group was more efficient than that of Vehicle group (Fig. 7A and 7B). And, KR group showed significantly reduced AUC compared with Vehicle group (Fig. 7C and 7D).

(A)


IPGTT (Initial)
Blood glucose (mg/dl)
900.0
800.0
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
Vehicle
KR
0
30
60
90
120
Time (min)

(B)


IPGTT (3 weeks later)
Blood glucose (mg/dl)
900.0
800.0
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
0
30
60
90
120
Time (min)

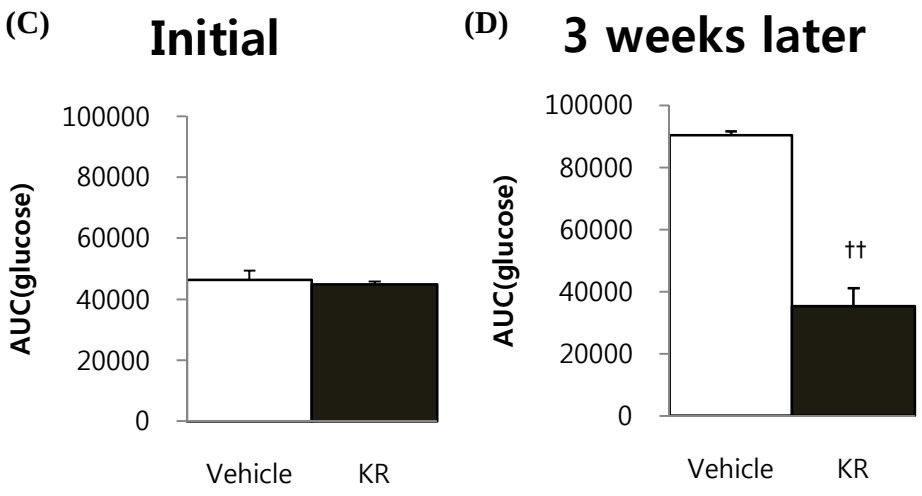


Figure 7. *ob/ob* mice administered KR-66195 for 3 weeks showed improved glucose tolerance during IPGTT. The mice administered KR-66195 for 3 weeks showed lowered fasting blood glucose level and effective glucose clearance ability during IPGTT (2g glucose/kg body wt). (A) At the initial IPGTT, similar glucose excursion profiles were shown. (B) But, 3 weeks later, KR group showed improved blood glucose tolerance and clearance ability than Vehicle group. (C) AUC for the IPGTTs on initial and (D) that on 3 weeks later were calculated from 0 to 120 min. Values are expressed as means ± SE; n=6 mice/group. ††, $P < 0.001$ vs. Vehicle.

6. KR-66195 increased plasma GLP-1 levels in *ob/ob* mice

After 3 weeks of treatment, blood was collected during sacrifice. 3 weeks treatment with KR-66195 significantly increased basal GLP-1 plasma concentration to 35.95 ± 14.06 pM in KR group, on the other hands, 6.58 ± 0.80 pM in Vehicle group (Fig. 8).

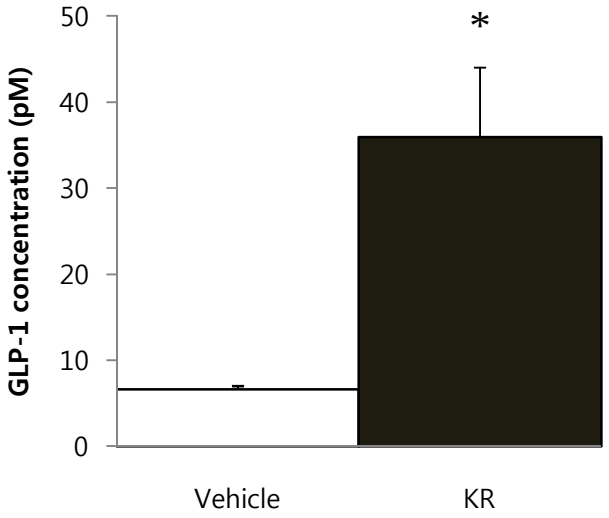


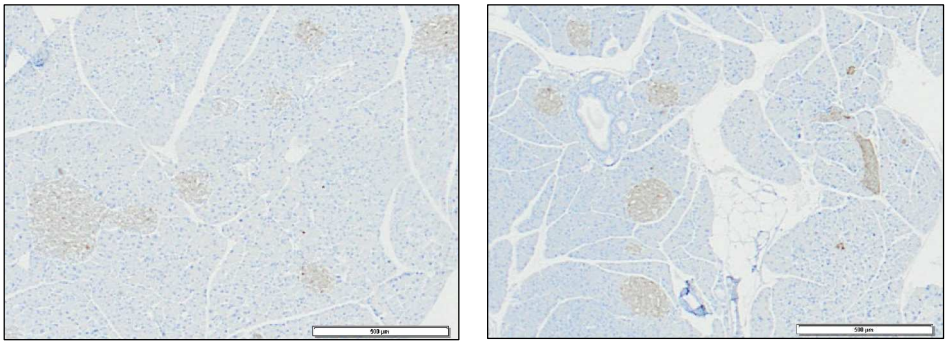
Figure 8. KR-66195 increased plasma GLP-1 concentration in *ob/ob* mice.

KR-66195 treatment for 3 weeks elevated plasma GLP-1 levels in ob/ob mice. Values are expressed as means ± SE; n=6 mice/group. *, $P < 0.05$ vs. Vehicle.

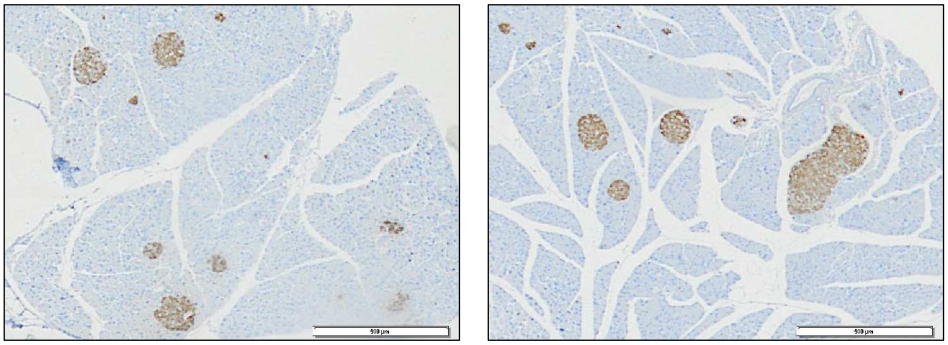
7. KR-66195 increased pancreatic insulin content in *ob/ob* mice.

After 3 weeks of treatment, the pancreas was isolated from each mouse and analyzed by immunohistochemistry using anti-insulin antibody. This analysis revealed decreased insulin staining in beta-cells of Vehicle group compared with KR group, indicating impaired beta-cell function in Vehicle group (Fig. 9A). In contrast, increased insulin staining in the beta-cells was observed in KR group compared with Vehicle group after 3 weeks of treatment (Fig. 9B). But, differences of area ratio of beta-cell to whole pancreas on pancreatic section slides were not significant.

(A)



(B)



(C)

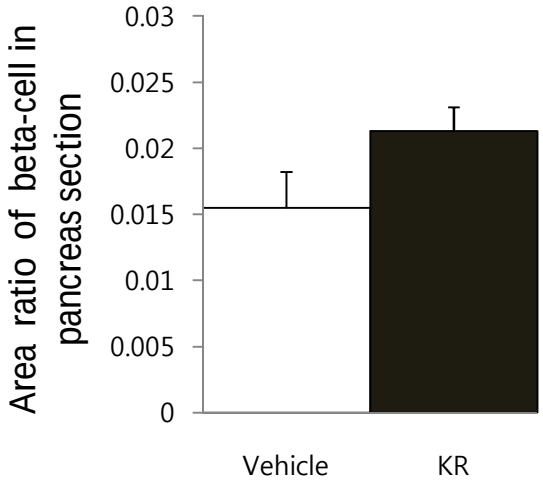

Area ratio of beta-cell in pancreas section
0.03
0.025
0.02
0.015
0.01
0.005
0
Vehicle
KR

Figure 9. Effects of KR-66195 treatment for 3 weeks on pancreatic insulin in *ob/ob* mice. After 3 weeks of KR-66195 treatment, pancreata were isolated and pancreatic sections were stained with anti-insulin antibody. (A) Vehicle group showed decreased insulin staining compared with KR group. (B) On the contrary, KR-group showed increased insulin staining. (C) But, differences of area ratio of beta-cell to whole pancreas on pancreatic section slides were not significant. Scale bar = 500 μm. Values are expressed as means ± SE; n=3 mice/group.

IV. DISCUSSION

In many studies, GLP-1 analogs, such as enxendin-4, and DPP-IV inhibitors showed similar effects on glucose control, but GLP-1 analog only showed decreased body weight.[27-30] The new DPP-IV inhibitor KR-66195 described in this article was able to inhibit circulating DPP-IV activity and therefore increase plasma GLP-1 concentration; it then effectively reduced food intake and body weight gain in diet-induced obese C57BL/6 and obese-diabetic *ob/ob* mice. Moreover, KR-66195 reduced the glucose excursion following IPGTT in C57BL/6 and *ob/ob* mice.

One key outcome in this study was that KR-66195 prevented body weight gain related with antiobesity effect in both in C57BL/6 mice and *ob/ob* mice. It could be explained with reduced food intake which was shown in our results. One study showed GLP-1 could make taste change or aversion by modulating taste sensitivity especially to sweet and sour taste.[31] GLP-1 is also known to reduce feeding through a brain originated peptide acting on hypothalamic GLP-1 receptors, causing hypothalamic anorectic effect.[32] And other gut hormones inactivated or activated by DPP-IV, such as peptide-YY or oxyntomodulin are likely reasons for their reduced feeding.[33-34] These findings could be reasons for reduced food intake and weight gain in our mice studies.

Interestingly, Studies have shown hyperglycemia elevated DPP-IV activity in patients with type 2 diabetes and in the obese diabetic *ob/ob* mice.[35-37] In

addition, the incretin effect is thought to be partly impaired due to a reduction in GLP-1 secretion and a reduction in the pancreatic response to GIP.[38-40] With these studies, we could predict why the effects of DPP-IV inhibition by KR-66195 that caused preventing weight gain and increasing GLP-1 concentration are more potent in *ob/ob* mice than those in C57BL/6 mice in our studies. Moreover, anorectic effect of KR-66195 was also more potent in *ob/ob* mice than that in C57BL/6 mice. The precise mechanisms underlying the effects of DPP-IV inhibition in *ob/ob* mice remain to be determined with more studies.

In addition, KR-66195 decreased epididymal fat ratio in C57BL/6 mice. KR-66195 administrated group showed significantly low epididymal fat ratio level. It was almost consistent with the level of CHD+vehicle group. One study showed DPP-IV inhibitor, vildagliptin, could improve postprandial plasma triglyceride and apolipoprotein B-48-containing triglyceride-rich lipoprotein particle metabolism after a fat-rich meal in patients with type 2 diabetes.[41] And, another study showed GLP-1 analog could reduce intestinal lymph flow, triglyceride absorption, and apolipoprotein production in rats.[42] Therefore, there could be several possible mechanisms by which KR-66195 could have mediated lowering fat accumulation and increasing lipid metabolism, as other GLP-1 analogs or DPP-IV inhibitors had shown. To elucidate precise mechanism, more studies about physical activities or fat metabolism need to be conducted.

KR-66195 treated mice showed improved glucose tolerance than the vehicle treated mice following the IPGTT. Furthermore, we could see increased pancreatic insulin staining in *ob/ob* study. So, the improvement of insulin secretion and efficacy, resulting in improved glucose tolerance observed in this study could have been mediated by enhanced GLP-1 secretion. Moreover it was occurred despite the complete lack of measurable plasma DPP-IV inhibition after 40 hours drug washout and before glucose challenge in C57BL/6 study. Taken together, these results suggest that chronic treatment with KR-66195 potently inhibited DPP-IV, improved beta-cell function and prevented insulin resistance in both C57BL/6 and *ob/ob* mice.

Furthermore, this study demonstrated that chronic inhibition of DPP-IV by KR-66195 could delay the occurrence of type 2 diabetes in diet-induced obese C56BL/6 and obese-diabetic *ob/ob* mice. Permanent inhibition of circulating DPP-IV activity most likely leads to a sustained action of GLP-1 at the pancreatic level. GLP-1 also stimulates increases in beta-cell mass and insulin synthesis and thus favors adequate insulin stores in pancreatic islets.[43-44] These findings are consistent with our results. It is therefore speculated that in the clinic, DPP-IV inhibitors such as KR-66195 could delay or even prevent obesity and the progression from impaired glucose tolerance to type 2 diabetes by improving glucose tolerance and preserving beta-cell function.

As summary, in the present studies, the effect of long term treatment with KR-66195 on metabolic control was investigated in diet-induced obese

C57BL/6 mice and that of short term treatment in obese-diabetic *ob/ob* mice. After 8 weeks of treatment in C57BL/6 and 3 weeks of treatment in ob/ob, KR-66195 potently inhibited plasma DPP-IV activity and increased plasma active GLP-1 levels, resulting in reduction of food intake, improved glycemic control and glucose tolerance and especially reducing body weight gain.

V. CONCLUSION

In conclusion, long term administration of KR-66195 resulted in increase in the amount of plasma GLP-1, improvement of glucose tolerance and reduction in body weight gain, epididymal fat accumulation and food intake in C57BL/6. And short term administration of KR-66195 consistently resulted in the comparible effects as well as increased pancreatic insulin content in *ob/ob* mice. These are meant that improved beta-cell function and antiobesity effects in C57BL6 and *ob/ob* mice, suggesting that KR-66195 may also exhibit these beneficial effects when administered to obese patients with type 2 diabetes or diabetes alone. Further development of stable DPP-IV inhibitor, such as KR-66195, might be new effective agent for antiobesity therapy as well as diabetes.

REFERENCES

1. Kumanyika S, Jeffery RW, Morabia A, Ritenbaugh C, Antipatis VJ. Obesity prevention: the case for action. Int J Obes Relat Metab Disord 2002;26:425-36.
2. Goldstein DJ. Beneficial health effects of modest weight loss. Int J Obes Relat Metab Disord 1992;16:397-415.
3. Wing RR, Marcus MD, Epstein LH, Salata R. Type II diabetic subjects lose less weight than their overweight nondiabetic spouses. Diabetes Care 1987;10:563-6.
4. Kelley DE, Wing R, Buonocore C, Sturis J, Polonsky K, Fitzsimmons M. Relative effects of calorie restriction and weight loss in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 1993;77:1287-93.
5. Kendall DM, Rubin CJ, Mohideen P, Ledeine JM, Belder R, Gross J, et al. Improvement of glycemic control, triglycerides, and HDL cholesterol levels with muraglitazar, a dual (alpha/gamma) peroxisome proliferator-activated receptor activator, in patients with type 2 diabetes inadequately controlled with metformin monotherapy: A double-blind, randomized, pioglitazone-comparative study. Diabetes Care 2006;29:1016-23.
6. Schernthaner G, Matthews DR, Charbonnel B, Hanefeld M, Brunetti

P. Efficacy and safety of pioglitazone versus metformin in patients with type 2 diabetes mellitus: a double-blind, randomized trial. J Clin Endocrinol Metab 2004;89:6068-76.

7. Lloret-Linares C, Greenfield JR, Czernichow S. Effect of weight-reducing agents on glycaemic parameters and progression to Type 2 diabetes: a review. Diabet Med 2008;25:1142-50.

8. Vilsboll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. Diabetes 2001;50:609-13.

9. Toft-Nielsen MB, Madsbad S, Holst JJ. Determinants of the effectiveness of glucagon-like peptide-1 in type 2 diabetes. J Clin Endocrinol Metab 2001;86:3853-60.

10. Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Bonner-Weir S, Habener JF, et al. Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. Diabetes 2000;49:741-8.

11. Perfetti R, Zhou J, Doyle ME, Egan JM. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. Endocrinology 2000;141:4600-5.

12. Meier JJ, Gethmann A, Nauck MA, Gotze O, Schmitz F, Deacon CF, et al. The glucagon-like peptide-1 metabolite GLP-1-(9-36) amide

reduces postprandial glycemia independently of gastric emptying and insulin secretion in humans. Am J Physiol Endocrinol Metab 2006;290:E1118-23.

13. Pannacciulli N, Le DS, Salbe AD, Chen K, Reiman EM, Tataranni PA, et al. Postprandial glucagon-like peptide-1 (GLP-1) response is positively associated with changes in neuronal activity of brain areas implicated in satiety and food intake regulation in humans. Neuroimage 2007;35:511-7.

14. Deacon CF, Ahren B, Holst JJ. Inhibitors of dipeptidyl peptidase IV: a novel approach for the prevention and treatment of Type 2 diabetes? Expert Opin Investig Drugs 2004;13:1091-102.

15. Mest HJ, Mentlein R. Dipeptidyl peptidase inhibitors as new drugs for the treatment of type 2 diabetes. Diabetologia 2005;48:616-20.

16. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet 2006;368:1696-705.

17. Pratley RE, Salsali A. Inhibition of DPP-4: a new therapeutic approach for the treatment of type 2 diabetes. Curr Med Res Opin 2007;23:919-31.

18. Gallwitz B. Exenatide in type 2 diabetes: treatment effects in clinical studies and animal study data. Int J Clin Pract 2006;60:1654-61.

19. Henness S, Keam SJ. Vildagliptin. Drugs 2006;66:1989-2001;

discussion 2-4.

20. Vilsboll T. Liraglutide: a once-daily GLP-1 analogue for the treatment of type 2 diabetes mellitus. Expert Opin Investig Drugs 2007;16:231-7.

21. Farilla L, Hui H, Bertolotto C, Kang E, Bulotta A, Di Mario U, et al. Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. Endocrinology 2002;143:4397-408.

22. Rolin B, Larsen MO, Gotfredsen CF, Deacon CF, Carr RD, Wilken M, et al. The long-acting GLP-1 derivative NN2211 ameliorates glycemia and increases beta-cell mass in diabetic mice. Am J Physiol Endocrinol Metab 2002;283:E745-52.

23. Tourrel C, Bailbe D, Lacorne M, Meile MJ, Kergoat M, Portha B. Persistent improvement of type 2 diabetes in the Goto-Kakizaki rat model by expansion of the beta-cell mass during the prediabetic period with glucagon-like peptide-1 or exendin-4. Diabetes 2002;51:1443-52.

24. Perfetti R, Hui H. The role of GLP-1 in the life and death of pancreatic beta cells. Horm Metab Res 2004;36:804-10.

25. Pospisilik JA, Stafford SG, Demuth HU, Brownsey R, Parkhouse W, Finegood DT, et al. Long-term treatment with the dipeptidyl peptidase IV inhibitor P32/98 causes sustained improvements in glucose tolerance, insulin sensitivity, hyperinsulinemia, and beta-cell glucose

responsiveness in VDF (fa/fa) Zucker rats. Diabetes 2002;51:943-50.

26. Pospisilik JA, Martin J, Doty T, Ehses JA, Pamir N, Lynn FC, et al. Dipeptidyl peptidase IV inhibitor treatment stimulates beta-cell survival and islet neogenesis in streptozotocin-induced diabetic rats. Diabetes 2003;52:741-50.

27. Mu J, Woods J, Zhou YP, Roy RS, Li Z, Zycband E, et al. Chronic inhibition of dipeptidyl peptidase-4 with a sitagliptin analog preserves pancreatic beta-cell mass and function in a rodent model of type 2 diabetes. Diabetes 2006;55:1695-704.

28. Raun K, von Voss P, Knudsen LB. Liraglutide, a once-daily human glucagon-like peptide-1 analog, minimizes food intake in severely obese minipigs. Obesity (Silver Spring) 2007;15:1710-6.

29. Raun K, von Voss P, Gotfredsen CF, Golozoubova V, Rolin B, Knudsen LB. Liraglutide, a long-acting glucagon-like peptide-1 analog, reduces body weight and food intake in obese candy-fed rats, whereas a dipeptidyl peptidase-IV inhibitor, vildagliptin, does not. Diabetes 2007;56:8-15.

30. Xu G, Stoffers DA, Habener JF, Bonner-Weir S. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. Diabetes 1999;48:2270-6.

31. Shin YK, Martin B, Golden E, Dotson CD, Maudsley S, Kim W, et al.

Modulation of taste sensitivity by GLP-1 signaling. J Neurochem 2008;106:455-63.

32. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, et al. A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 1996;379:69-72.

33. Eberlein GA, Eysselein VE, Schaeffer M, Layer P, Grandt D, Goebell H, et al. A new molecular form of PYY: structural characterization of human PYY(3-36) and PYY(1-36). Peptides 1989;10:797-803.

34. Mentlein R, Dahms P, Grandt D, Kruger R. Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV. Regul Pept 1993;49:133-44.

35. Mannucci E, Pala L, Ciani S, Bardini G, Pezzatini A, Sposato I, et al. Hyperglycaemia increases dipeptidyl peptidase IV activity in diabetes mellitus. Diabetologia 2005;48:1168-72.

36. Meneilly GS, Demuth HU, McIntosh CH, Pederson RA. Effect of ageing and diabetes on glucose-dependent insulinotropic polypeptide and dipeptidyl peptidase IV responses to oral glucose. Diabet Med 2000;17:346-50.

37. Korosi J, McIntosh CH, Pederson RA, Demuth HU, Habener JF, Gingerich R, et al. Effect of aging and diabetes on the enteroinsular axis. J Gerontol A Biol Sci Med Sci 2001;56:M575-9.

38. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt

W. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest 1993;91:301-7.

39. Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, et al. Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. J Clin Endocrinol Metab 2001;86:3717-23.

40. Meier JJ, Hucking K, Holst JJ, Deacon CF, Schmiegel WH, Nauck MA. Reduced insulinotropic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. Diabetes 2001;50:2497-504.

41. Matikainen N, Manttari S, Schweizer A, Ulvestad A, Mills D, Dunning BE, et al. Vildagliptin therapy reduces postprandial intestinal triglyceride-rich lipoprotein particles in patients with type 2 diabetes. Diabetologia 2006;49:2049-57.

42. Qin X, Shen H, Liu M, Yang Q, Zheng S, Sabo M, et al. GLP-1 reduces intestinal lymph flow, triglyceride absorption, and apolipoprotein production in rats. Am J Physiol Gastrointest Liver Physiol 2005;288:G943-9.

43. Sebokova E, Christ AD, Boehringer M, Mizrahi J. Dipeptidyl peptidase IV inhibitors: the next generation of new promising therapies for the management of type 2 diabetes. Curr Top Med Chem

2007;7:547-55.

44. Fridolf T, Bottcher G, Sundler F, Ahren B. GLP-1 and GLP-1(7-36) amide: influences on basal and stimulated insulin and glucagon secretion in the mouse. Pancreas 1991;6:208-15.

<ABSTRACT(IN KOREAN)>

식이 비만 유발 생쥐 (C57BL/6) 와 비만 당뇨 생쥐 (*ob/ob*) 에서 dipeptidyl peptidase-IV 저해재인 KR-66195 의 항비만 효과

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김 연 욱

그 동안 다양한 dipeptidyl peptidase (DPP)-IV 저해재들이 개발되었고 제2형 당뇨병 환자들의 혈중 active glucagon like peptide (GLP)–1의 농도를 올림으로써 향상된 glycemic control 효과를 보여주었다. 하지만 이들 DPP-IV 저해재들이 체중의 증가를 막는 효과는 보이지 않았다. 이번 연구를 통해 우리는 새로 개발된 KR-66195가 glycemic 조절 능력을 향상시킬 뿐 아니라 다른 DPP-IV 저해재들이 보여주지 못했던 항 비만 효과를 가지고 있다는 것을 식이 비만 유발 생쥐 (C57BL/6) 와 비만 당뇨 생쥐 (*ob/ob*) 실험을 통해서 밝혔다. 식이 비만 유발 생쥐에 KR-66195을 intraperitoneal

injection 방법으로 매일 8주간 10 mg per kg body weight 농도로 주사했을 때, 혈중 GLP-1의 농도가 증가 하고 향상된 glucose tolerance 효과를 보이고 epididymal 지방 조직의 크기와 먹이 섭취량을 감소 시켰으며 체중의 증가를 둔화 시켰다. 그리고 유전적으로 비만을 일으키는 비만 당뇨 생쥐에 KR-66195을 위의 실험에서와 같은 방법으로 3주간 주사했을 때, 식이 비만 유발 생쥐 실험과 유사한 효과들을 보이고 췌장 내부의 insulin 양이 증가하는 것을 확인할 수 있었다. 그러므로 앞으로 KR-66195가 더욱 연구, 보완되어 당뇨병뿐만 아니라 항비만 약제로써 쓰여질 수 있기를 기대한다.

핵심되는 말 : 비만, 당뇨, KR-66195, glucagon like peptide (GLP)-1, dipeptidyl peptidase (DPP)-IV, 체중 감소, *ob/ob* , 생쥐